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# EARTHWORMS AS ECOTOXICOLOGICAL ASSESSMENT TOOLS

by

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16. ABSTRACT

Increased interest for earthworm research and the need for soil assessment methods has encouraged the use of earthworms as assessment organisms. Earthworms exhibit many advantages for use in assessing the impact of toxic and hazardous materials on soil systems. Earthworms are known to accumulate heavy metals, pesticides and other organic chemicals supporting their importance as a model for assessing the general impact to the soil community. Laboratory studies have shown impacts on individual growth and reproduction by metals and organic chemicals. Although rigorous field studies are fewer in number, some have shown that edaphic and environmental factors affect densities of some species. Laboratory and field research to date strongly support the utility of earthworms for making assessments of ecological impacts associated with toxic agents. Earthworms are presently being evaluated by the United States Environmental Protection Agency as tools for assessing the impact of toxic and hazardous wastes on soil systems. Adverse effects of environmental samples from industrial waste disposal sites which contain pesticides can be detected by earthworms 14 Day Soil Test with as little as 2 percent of the parent soil sample when the 48 Hour Contact Test using water extracted leachates did not show any mortality. The assessment of soil and leachate samples using nerve transmission rate measurements generally corroborate mortality end points of the 14 Day Soil Test.

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## ABSTRACT

Increased interest for earthworm research and the need for soil assessment methods has encouraged the use of earthworms as assessment organisms. Earthworms exhibit many advantages for use in assessing the impact of toxic and hazardous materials on soil systems.

Earthworms are known to accumulate heavy metals, pesticides and other organic chemicals supporting their importance as a model for assessing the general impact to the soil community. Laboratory studies have shown impacts on individual growth and reproduction by metals and organic chemicals. Although rigorous field studies are fewer in number, some have shown that edaphic and environmental factors affect densities of some species. Laboratory and field research to date strongly support the utility of earthworms for making assessments of ecological impacts associated with toxic agents.

Earthworms are presently being evaluated by the United States Environmental Protection Agency as tools for assessing the impact of toxic and hazardous wastes on soil systems. Adverse effects of environmental samples from industrial waste disposal sites which contain pesticides can be detected by earthworm 14 Day Soil Test with as little as 2 percent ($LC_{50}$ = 1.8%) of the parent soil sample when the 48 Hour Contact Test using water extracted leachates did not show any mortality. The assessment of soil and leachate samples using nerve transmission rate measurements generally corroborate mortality end points of the 14 Day Soil Test.

Methods for use of earthworms in short term toxicity tests are well established, however the use of earthworms in longer term ecological assessments are less refined. Bioassay methods appear to have certain advantages in assessment protocols for hazardous wastes compared to chemical analysis alone.

## INTRODUCTION

The concerns for the impact of toxic and hazardous materials on the soil environment have continued to increase in recent years and, correspondingly, there have been accelerated efforts in developing methods for evaluation. Most of these methods have involved assessment of short-term effects primarily of human health (Goyer, 1983). There is, however, an equally important

need for assessing long-term toxic impacts on the terrestrial ecosystem, in which humans are considered but one of many components.

This paper reviews (1) the relevant literature concerning earthworms as ecological tools for assessment of the impact of toxic and hazardous wastes; (2) methods used in past research for measurement of the impact on earthworms at the individual, population and ecological levels; and (3) present efforts by the Corvallis Environmental Research Laboratory to use earthworms as assessment organisms to measure impact on soil systems.

This discussion is based on the premise that to assess the impact of chemicals one must determine the exposure of the chemical toxicant to the organism of interest and then determine the effect (Morarity, 1983a).

## DEFINITIONS

A toxic substance or pollutant is defined as a substance introduced to the environment by man that has a deleterious impact on biological organisms (Moriarty, 1983a). Ecotoxicology is the branch of toxicology which studies the ecological effects of toxic substances or pollutants (Truhant, 1975).

Ecotoxicological research is specifically concerned with biological problems at the level of populations and communities as they interact with the physical environment. However, the effects of pollutants and toxic materials are often mediated through their impact on individuals and the severity depends upon the dose (Moriarty, 1983a).

## EXPOSURE AND ACCUMULATION

Earthworms are a diverse group, they are essentially cosmopolitan given sufficient moisture and adequate temperatures. Because of their distribution, they are vulnerable to most insults subjected to the soil ecosystem especially associated with the application of agricultural chemicals (Wheatley and Hardman, 1968; Thompson, 1970; Randell et al, 1972).

There have been many efforts to show the impact of toxicants on earthworms with varying success. These studies have shown that earthworms generally respond to many chemicals and other factors in many ways (Roberts and Dorough, in press). Impacts on individuals can occur as increases in body burdens, increased mortality and overall decrease in activities normally associated with viable earthworm populations (Edwards and Thompson, 1973).

Decreases in numbers as well as impacts on their life activities such as feeding e.g., vegetation processing (Barker, 1958) has been the subject of research in assessment of chemical impacts to earthworms (Edwards and Thompson, 1973). Although there are reports of field evaluations of earthworm impacts, these are fewer than laboratory studies. The methods for field studies appear to be more varied and represent considerable

questions as to the value of the results for assessment in an ecological sense (Lofs-Holmin, 1984). There are reported problems in assessing density, total numbers, application rates of toxicants, concentrations of toxicants in the field, as well as interpretation of the meaning of the results for many of these studies (Roberts and Dorough, in press). Summarization of these research reports is difficult because of the problem of trying to collate the diverse methods under as many objectives used for the studies resulting in difficulties in interpretation of the data.

## EFFECTS ON THE INDIVIDUAL

Toxic impacts on individual earthworms will affect the population as reductions in numbers as well as removal of a portion of the gene pool. Assessments of exposure for heavy metals is primarily through measurements of body burden (Ireland and Richards, 1981; Carter et al, 1981; Edwards and Lofty, 1977);(Gish,, 1970).

Growth and reproduction has been influenced in the laboratory by heavy metals (Malecki, et al, 1982) and these characteristics should be affected under field conditions. Changes in densities noted in past studies (Raw, 1962) should be further investigated to determine the dose-response mechanism or other important factors related to changes in numbers. Recent research of the dynamics of contaminants emphasizes the complex nature of the interrelationships encountered in studying heavy metals in earthworms (Ma, 1982; Ma et al, 1983; and Ma, 1984). Cobolt has been shown to increase the maximum weight of E. fetida but also causes a lag in early growth (Neuhauser et al, 1984a). Neuhauser et al, (1984b) exposed E. fetida to several heavy metal salts to show that this species is capable of compensatory growth whereas it did not compensate for reproduction. E. fetida, however, was shown to be quite resilient resuming normal growth and reproduction after the worms were removed from impacted soil.

Pesticides and herbicides at the individual level have been assessed as qualitative decrease in numbers (Ruppel and Laughlin, 1977). Although some of these studies considered changes in density and species composition none rigorously examined any ecological variables such as community structure and function (Farnworth and Golley, 1979). There is little imformation relating soil concentration to sublethal effects such as reduced reproduction (Lebrun, et al, 1981 and DeMedts, 1981), predator-prey relationships, fecundity, changes in egg fertility, growth and differential mortality with age or changes in behavior. There needs to be more evaluation of the dose-response relationship under field conditions (Moriarty, 1983a) incorporating a study of those factors important to changes in density, growth, reproduction, and mortality.

## EFFECTS ON POPULATIONS

Ecologically, the impact of toxic materials is at the population level (Moriarty, 1983b). Although the acute impacts

immediately remove certain individuals from the breeding population, change in numbers (i.e. population size) is the most obvious impacts that can be assessed. Population fluctuations in numbers are controlled by birth rates, death rates, gains from immigration and losses from emigration. Measurement of these four factors can be used to determine the changes in population size (Moriarty, 1983a). There are few if any field studies with good quantitative data on changes in birth rates and death rates (Lebrun et al, 1981, De Medts, 1981). Studies on population changes due to environmental factors are more plentiful and include measured impact of pH on individual growth (Satchell,1967) and death (Southwell and Majer, 1982) and temperature (Standen et al, 1982) and immigration due to low oxygen and high carbon dioxide concentrations. (Schriefer, 1981).

In summary, past research has shown that earthworms accumulate heavy metals, pesticides as well as other organic chemicals affecting their growth, reproduction and mortality at the individual level. Although population level impacts are more difficult to documunt, decreases in numbers, population density, birth rate, and immigration rate are suspected to occur under field conditions. There has been very little research concerning ecological impact of toxicants especially any measuring community structure impact, predator-prey relationships or parasite-host effects. The above discussion serves as a description of the immense problems encountered in efforts to move from the single species, individual organism evaluation through the population level to a more complex multispecies ecological level of earthworm assessments.

## EARTHWORMS AS AN ASSESMENT TOOL FOR TOXIC AND HAZARDOUS WASTES

Presently the Hazardous Materials Assessment Team (HMAT) is evaluating earthworm bioassays (48 Hour Contact Test, the 14 Day Soil Test and neurophysiological impact) to assess the toxicity of chemicals to soil systems (Porcella, 1983).

In the 48 Hour Contact Test (CT) (Goats and Edwards, 1982) the chemical being evaluated is dispersed in water or an organic solvent depending upon its solubility characteristics and pipetted into filter paper lined glass vials. A residue of the chemical remains on the filter paper when the solvent is evaporated. Water is used to moisten the paper before placing one worm into the vial which is then capped. The vials are stored on their side in the dark for 48 hours afterwhich they are assessed for mortality. The concentration is expressed as ug/cm2 of the chemical on the filter paper lining the vial or as ug/ml.

The 14 Day Soil Test (ST) (Goats and Edwards, 1982) involves mixing the chemical of interest in a standard mix of sand, clay and peat. Moisture is added to 35% by weight and usually 10 earthworms are placed into 400 grams of the mixture. Initial and final weights after 14 days allows one to monitor changes in weight as well as mortality with concentration. Chemical concentration is expressed as ug/g of soil.

The neurological assay is an assessment of nerve transmission rates (NTR) as related to concentration of chemical and is made with either of the exposure methods described above (Drewes, et. al, 1983) NTR measurements are made before, during, and after the exposures to monitor the changes as affected by the toxicant.

Thus far our experience with samples from various industrial waste sites has shown that (1) the CT, as expected, is effective in detecting toxicity when the toxicant is water soluble; and (2) the ST is effective in indicating toxicity of various toxicants (both water soluble and non water soluble), including organic chemical pesticides and a mixture of metals, solvents and polynuclear aromatic hydrocarbons. For example, an industrial sample where there was no mortality observed for the CT, the $LC_{50}$ for the ST was less than 2% of site soil (w/w). This sample contained high concentrations of dieldrin, endrin, and aldrin. These results are indicative of the difference between the CT and the ST. Also, preliminary NTR measurements of this sample leachate (water soluble portion) show significant diffferences compared to distilled water after exposure of only 15 minutes. Nerve transmission rate measurements have been shown to be very effective in picking up toxicities at lower concentrations and shorter exposures (Drewes and Vining, 1984). For instance the dieldrin LC 50 by CT measurements are at 8100 ppm whereas there was significant NTR differences at 1.4 ppm within 2 hours of exposure.

Bioassay results can be used to interpret the impact of toxicants in two ways: 1) toxicity evaluation and 2) ecological impact. The earthworm bioassays described above are sufficient to determine the relative toxicity of chemicals to earthworms (Drewes, 1984; Roberts and Dorough, 1984) but as yet have limited if any known ecological significance. The present interpretation of CT results is limited by the application of the test. For example, in the CT interpretation of toxicity, results, are limited to the relative toxicity of reported standards or data established for that chemical (Goats and Edwards, 1982). That is, the tests are performed in the laboratory and results are compared to known standards also derived from laboratory results. Thus, the reliance on laboratory results is the chief limitation toward field application.

The ST is limited at this time because the tests results have no ecological correlates as they are presently applied. The endpoints measured in the laboratory have not been shown conclusively to have ecological significance by measurement in the field. Of course, the tests could theoretically be extended to a field interpretation e.g., mortality, reduced growth and reproduction, and changes in physiological functioning with well designed field experiments. This limitation induces us to better define the use of the endpoints and to recognize the limited relationship between the laboratory results and the field evaluations that may contain ecological relevance.

The alternative to bioassay assessment, chemical analysis is inadequate when practiced alone and therefore unacceptable under most conditions (Lysyj, 1981). For instance, when unknown chemicals are encountered during an assessment activity, the most widely used method now practiced is to perform chemical analysis for identification and concentration levels of EPA priority pollutants. Our experience (and many others apparently) with many field samples is that frequently we encounter chemicals in mixtures with little known history and no known toxicity levels. Reality is that upon chemical analysis there are many unidentified chemicals in solution for which we have no known toxicological knowledge (Unrweberg et al, 1980). There is a distinct advantage in performing bioassay tests under these circumstances because at minimum one has an estimate of the bioavailability of the complex mixture even when it can't be characterized chemically. The bioassay information can be used to arrive at an acute and hopefully sublethal toxicity measurement which in turn, can be used for guidance in solving the waste problem (Schweitzer, 1981).

For assessment of sites with limited exposure where the toxicants are under confinement or are not widespread the need is clearly one of toxicity testing. The most immediate need is to determine the actual danger of the chemicals in terms of acute toxicity. The CT and the ST (and possibly the NTRT under evaluation) may be used without hesitation in the first example to determine relative toxicity. Test results can be interpretated with respect to known standards and to other studies that have examined similar chemicals and situations. The evaluation of acute and relative toxicity can be made without extrapolations or extentions of the data set beyond the ranges of experience.

When a situation is encountered where the exposure is widespread or uncontrollable, the need is not only one of toxicity determination, but also one of potential ecological impact (Reinecke and Venter, 1984). This situation is clearly a more difficult one to assess because of the need for more comprehensive information. The above acute toxicity tests appear to be inadequate for ecological assessment as currently used. Evaluations of reduced growth and reproduction (Lofs-Holmin, 1980, 1982), as well as impacts on neurophysiology (Drewes et al,1984), show promise in assessment of longer term and more complex impacts.

In an attempt to overcome the problems of limited interpretation we are redesigning our experiments for measuring effects in the laboratory that will have more relevance in the field. For instance, sublethal measurements such as growth, reproduction, and neurophysiological changes are planned for the laboratory and field at the same level of exposure. In this way the concentration (i.e. exposure) of the chemical in the organism (i.e. body burden) is the connection to the effect that we would expect to see in the field. If environmentally observed concentrations can be duplicated in the laboratory in terms of

body burdens we can make more credible predictions of changes in growth and reproduction and ecological impact. We are using evaluation procedures that should improve our ability to determine the measurable effects in the field. To do this, we are bringing field samples to the laboratory for bioassay evaluation and chemical analysis. These laboratory procedures do not duplicate the field experiments, but do provide data that can be interpretated in terms of risk. These procedures represent a compromise in overcomming the tremendous problems of performing a full field experiment that may be cost prohibitive and can be inconclusive when completed.

The CT and the ST appear to be adequate for toxicity assessment of pollutants in hazardous wastes whereas they have limited interpretation for ecological impact. However, modifications of the ST and well defined field tests should enable an ecological assessment for sublethal effects of growth, reproduction and neurophysiological functioning. Cooperative research with Cornell and Iowa State Universities is underway to investigate the field concentrations that impact growth, reproduction and density of natural populations.

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